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Monitoring Innate and Adaptive Immune Markers in Rats During Experimental *Salmonella* Spp. Sepsis

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Abstract **Background:** Sepsis caused by *Salmonella* spp. represents a critical public health challenge due to its potential to cause severe systemic inflammation, multi-organ failure and high mortality rates. Despite advances in medical research, the precise immunological mechanisms driving sepsis remain poorly understood. Investigating the interplay between innate and adaptive immune responses during bacterial sepsis is essential for identifying novel therapeutic strategies to mitigate inflammation and immune dysregulation. **Objective:** This study aimed to investigate the innate and adaptive immune responses in rats during Salmonella spp. sepsis. **Methods:** We monitored antibody production, immune cell populations and inflammatory cytokine levels throughout the course of infection in a rat model. **Results:** Our findings revealed a transition from innate to adaptive immunity during the early phase of sepsis. This phase was characterized by increased levels of pro-inflammatory cytokines, neutrophils and macrophages. Adaptive responses, including antibody production, followed this initial activation. **Conclusion:** These results provide valuable insights into the immunological shifts during bacterial sepsis, offering a basis for potential therapeutic interventions targeting immune modulation.

Key Words Sepsis, *Salmonella* spp., Innate Immunity, Adaptive Immunity, Immune Response

INTRODUCTION

Sepsis, a potentially lethal condition, develops when the host immunological response to infection is dysregulated. If untreated, numerous organs may fail, causing death. Globally, sepsis kills 11 million people each year [1]. *Salmonella* spp., intestinal and invasive pathogens, are a significant sepsis pathogen [2].

Salmonella infections generally cause food poisoning but the organism may also enter the circulation and cause septicemia and sepsis [3]. The human immune system fights *Salmonella* spp. Infections in the blood once they penetrate the intestinal barrier. The immune system's innate and adaptive components mediate this response [4]. The innate immune system uses dendritic cells, macrophages and neutrophils to recognize and respond to pathogen Pattern Recognition Receptors (PRRs) [5]. Producing pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β triggers a cascade of mechanisms inhibiting the infection. Sepsis symptoms, such as vasodilation, increased vascular permeability and tissue damage, may result from immune activation and severe systemic inflammation [6].

The adaptive immune response occurs when an infection worsens. T and B cells are key to this pathogen-specific immune system branch. Key participants in cellular immunity orchestration are CD4+ helper and CD8+ cytotoxic T lymphocytes. B cells produce antigen-specific antibodies for humoral immunity [7].

Despite substantial research, the immunological mechanisms underpinning sepsis, particularly the complex interplay between innate and adaptive immunity, remain incompletely understood. Sepsis is often described as a "double-edged sword," [8]. Where the immune response is both protective and pathological. In the early stages, the immune system attempts to control the infection. Still, persistent immune activation can result in immune paralysis, characterized by lymphocyte exhaustion, impaired antigen presentation and increased susceptibility to secondary infections [9,10].

Animal models of sepsis have been pivotal in advancing our understanding of the immune responses during sepsis. Sepsis research often uses rats because they have comparable immune system responses to humans [11]. We tracked dynamic changes in innate and adaptive immunological markers in a *Salmonella* spp.-sepsis rat model. We studied cytokine levels, immune cell populations and antibody generation at different phases of Salmonella infection to better understand the immunological response. These findings may reveal treatment targets to enhance septic patient outcomes.

METHODS

Experimental Animals

Male Sprague-Dawley rats, weighing between 220 and 250 grams, were obtained from a certified animal facility. The rats were housed in a controlled environment with a 12 hour light/dark cycle, maintaining consistent temperature and humidity conditions. Standard laboratory chow and water were provided *ad libitum* to ensure proper nutrition and hydration. All experimental procedures were conducted in compliance with the ethical guidelines set by the Institutional Animal Care and Use Committee (IACUC), with prior approval obtained to ensure adherence to animal welfare standards [12].

Induction of Sepsis

Sepsis was induced by intraperitoneal injection of 1×10^8 CFU of *Salmonella* enterica serovar Typhimurium (ATCC 14028) in 500 µL of sterile Phosphate-Buffered Saline (PBS). Control rats received a PBS injection without bacteria. Animals were monitored for clinical signs of sepsis, including lethargy, piloerection and weight loss. Rats were sacrificed at specified time points (6 hours, 24 hours, three days, seven days) post-infection to analyze immune markers [13].

Immune Marker Assessment

Cytokine Measurement: Serum levels of TNF-α, IL-6, interleukin-10 (IL-10) and interferon-gamma (IFN-γ) were measured using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's protocols. Serum samples were collected at each time point and analyzed using a microplate reader at 450 nm [14].

Cellular Immune Response

Flow cytometry was used to assess the populations of innate and adaptive immune cells, including neutrophils, macrophages, CD4+ T cells, CD8+ T cells and B cells. Spleens were harvested from each rat and splenocytes were isolated and stained with fluorochrome-conjugated antibodies targeting specific surface markers (CD11b for macrophages, CD3 for T cells, CD19 for B cells). Data were acquired on a flow cytometer and analyzed using FlowJo software [15].

Antibody Measurement

Anti-Salmonella antibodies were measured using ELISA. Blood samples were collected and serum was separated and diluted before being plated on ELISA plates coated with Salmonella antigen. Bound antibodies were detected using horseradish peroxidase (HRP)-conjugated secondary antibodies and absorbance was measured at 450 nm [16].

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (Version X). Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. p-values of <0.05 were considered statistically significant.

RESULTS

Cytokine Profiles During Sepsis

The study revealed notable changes in cytokine levels during Salmonella spp. sepsis, with elevated pro-inflammatory markers (TNF-α, IL-6 and IFN-γ) detected as early as 6 hours after infection. TNF-α reached its peak at 24 hours; however, IL-6 remained persistently elevated for three days, suggesting a strong systemic inflammatory response. Although the anti-inflammatory cytokine IL-10 exhibited a delayed increase, it ultimately reached its peak levels by day 7, reflecting a compensatory mechanism aimed at reducing inflammation. Statistical analysis (*$p<0.05$, *$p<0.01$) validated these findings as significant when compared to normal baseline values. This indicates a dynamic immune response, transitioning from acute inflammation to regulatory mechanisms, which are vital in influencing the outcome of sepsis (Table 1).

Innate Immune Cell Populations

Flow cytometric analysis showed that the number of innate immune cells was significantly increased in Salmonella sepsis. Neutrophil levels increased significantly, peaking at 24 h post-infection (35.7±2.5%), then decreased but remained elevated until day 7 compared to control. The number of macrophages showed a continuous increase, reaching a peak (20.5±1.8%) on day 3 and still significantly higher than the control group on day 7. These findings are consistent with high levels of cytokines such as TNF-α and IL-6, indicating strong stimulation of the immune system in early bacterial sepsis (Table 2).

Adaptive Immune Response

The investigation of T and B cell populations has revealed that adaptive immune responses were initially suppressed during the early phase of *Salmonella* spp. Sepsis-likely (due to) the overwhelming inflammatory environment. CD4+ and CD8+ T cell populations experienced a significant reduction at 6 and 24 hours post-infection; this is consistent with the early dominance of the innate immune response. However, by day 3, T cell populations began to recover. Furthermore,

Table 1: Serum Cytokine Profiles in Rats During Experimental Salmonella-Induced Sepsis (pg/mL)

Time Post-Infection	TNF-α	IL-6	IL-10	IFN-γ
6 Hours	320±30	280±25	90±15	110±20
24 Hours	560±45**	500±40**	180±20*	340±40**
3 Days	450±35**	480±35**	210±25**	290±35**
7 Days	200±20*	310±30*	300±35**	160±25

Values are presented as Mean±SD. *p<0.05, **p<0.01 compared to controls

Table 2: Percentage of Innate Immune Cells in Spleen

Time Post-Infection	Neutrophils (%)	Macrophages (%)
Control	10.5±1.5	8.2±0.8
6 Hours	25.2±2.0**	15.5±1.2**
24 Hours	35.7±2.5**	22.4±2.0**
3 Days	30.8±2.2**	20.5±1.8**
7 Days	20.1±1.8**	18.6±1.5*

Data are expressed as Mean±Standard deviation (SD), Statistical significance compared to control: *p<0.05, **p<0.01

Table 3: T and B Cell Populations in Spleen

Time Post-Infection	CD4+ T Cells (%)	CD8+ T Cells (%)	B Cells (%)
Control	38.5±2.2	21.3±1.8	17.8±2.0
6 Hours	30.2±1.5*	15.0±1.2*	15.2±1.5
24 Hours	25.5±2.0*	13.8±1.0*	13.5±1.2*
3 Days	32.1±2.3	18.0±1.5	18.6±2.0
7 Days	42.8±3.0**	23.5±2.2**	22.0±2.5*

Table 4: Serum Antibody Levels (OD 450 nm)

Time Post-Infection	IgM	IgG
Control	0.12±0.02	0.08±0.01
6 Hours	0.15±0.03	0.09±0.02
24 Hours	0.18±0.04	0.10±0.02
3 Days	0.35±0.05**	0.25±0.03*
7 Days	0.60±0.06**	0.55±0.05**

a notable increase in CD4+ T cells was observed by day 7 (42.8±3.0%), indicating the activation of the adaptive immune response. B cell populations, however, followed a delayed activation pattern, with a substantial increase by day 7, correlating with the peak in antibody production. These results suggest that although the adaptive immune system is initially hindered, it eventually mounts a robust response to combat the infection because of these underlying dynamics (Table 3).

Antibody Response

The antibody response to Salmonella spp. infection exhibited a delayed pattern; this is particularly evident when juxtaposed with the swift activation of the innate immune system. Anti-Salmonella IgM antibodies were detectable by day 3 post-infection (reflecting) an initial phase of adaptive immune response. Notably, IgM levels significantly increased by day 7 (0.60±0.06), indicating a primary response. Conversely, IgG antibodies, which are indicative of a secondary immune response, began to rise at day 3 (0.25±0.03) and peaked by day 7 (0.55±0.05); thereby, suggesting the transition from primary immune response to a more robust, long-lasting secondary immune response. However, although these findings support gradual activation and maturation of the adaptive immune response over the course of infection, they raise questions regarding underlying mechanisms involved in this complex interplay (Table 4).

DISCUSSION

Sepsis is a complex reaction to infection characterized by dysfunction of the immune system leading to severe inflammation and reduced immunity. In this study, we investigated the immune response during Salmonella-induced bacterial sepsis in mice, focusing on inflammatory cytokine levels, immune cell activity and immunity.

The early stages of sepsis are characterized by a strong immune response, characterized by increased levels of proinflammatory cytokines such as TNF-α, IL-6 and IFN-γ, which rise within 24 hours of infection.

These findings are consistent with previous research showing that these cytokines play an important role in triggering the inflammatory response during bacterial infection. TNF-α is an important cytokine associated with sepsis due to its role in vascular damage, hypotension and organ failure [17]. Likewise, IL-6 is considered to be a factor that plays a central role in the inflammatory response by activating several immune cells [18]. Although these cytokines are important for disease control, excessive production can damage tissue and worsen the disease. Our findings show that TNF-α and IL-6 levels are elevated early in sepsis, highlighting their important role in initiating inflammation in sepsis [19].

In addition to cytokine production, innate immune cells such as neutrophils and macrophages also proliferate in the spleen during primary infection. Neutrophils are known to eliminate microorganisms by phagocytosis and release of

antimicrobial peptides, which peak within 24 hours, as previously reported [20]. Macrophages, on the other hand, continue to proliferate during infection, suggesting that they play an important role in the management of inflammation and sepsis. This observation is consistent with the dual role of macrophages in pathogen clearance and inflammation regulation [21,22].

Despite the strong innate immune response, immune suppression is a hallmark of sepsis, as demonstrated in our study by elevated IL-10. As the disease progresses, IL-10 levels increase dramatically. IL-10 is a cytokine known for its immunomodulatory actions by inhibiting the production of inflammatory cytokines and preventing severe inflammation [23]. This late-phase increase in IL-10 is essential for maintaining immune homeostasis but downregulation of this pathway leads to secondary infection or immune paralysis [24]. Therefore, the timing and levels of IL-10 are important for maintaining immune balance and preventing severe inflammation and immunosuppression during sepsis [25].

For adaptive immunity, responses were delayed but important for pathogen clearance and long-term resistance. In our study, both CD4+ and CD8+ T cells showed a prolonged response, with CD4+ cells surviving on day 3 and peaking on day 7 after infection. This delayed T-cell response is known as "fatigue," a common feature of sepsis [26]. Recovery of CD4+ cells (required to mount an immune response) and CD8+ cells (required to kill infected cells) is important to restore immune function during sepsis [27]. B cells, which play an important role in the production of antibodies to eliminate pathogens, also showed a delayed response, with IgM levels detected on day 3 and IgG levels on day 7. This delay in antibody production is consistent with slow activation of the adaptive system. immune system in sepsis. An increase in IgG levels indicates a shift from an immune response to a specific and effective immune response [28,29].

These findings are therapeutic. Current treatments for sepsis focus on controlling the infection with antibiotics, supporting organ function and managing fluid balance. However, understanding the role of immunity and inflammation in sepsis paves the way for therapeutic interventions that may improve patient outcomes. Our findings suggest that targeting inflammatory cytokines during the early stages of the disease may help reduce the negative effects of acute inflammation [30]. On the other hand, strategies aimed at improving the activity of T and B cells in the later stages of sepsis may improve recovery and reduce the risk of complications [3].

CONCLUSIONS

This study provides a comprehensive analysis of the immune response during Salmonella sepsis, focusing on the important role of inflammatory cytokines, innate immune cells and adaptive immunity in microbiological clearance and resolution of sepsis. These results highlight the importance of controlling pathogens by modulating immune responses early in disease and the need to restore adaptive immunity to fight disease and promote long-term disease. These findings contribute to our understanding of the pathogenesis of sepsis and provide guidance for the development of immunomodulatory therapies for patients with sepsis.

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